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Ceftriaxone attenuates glutamate-mediated neuro-inflammation and restores BDNF in MPTP model of Parkinson's disease in rats

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ABSTRACT

The present study is designed to investigate the role of glutamate transporter in neuroprotection of ceftriaxone against MPTP induced PD animal model. Young male Wistar rats were subjected to intra-nigral administration of MPTP for the induction of Parkinson's disease. Glutamate modulators like ceftriaxone (CFX), Memantine (MEM) and Dihydrokainate (DHK) were administered to MPTP-lesioned rats. Different behavioral alterations were assessed in between the study period. Animals were sacrificed immediately after behavioral session, and different biochemical parameters were measured. Intranigral administration of MPTP showed significant impairment of motor behavior and marked increase in inflammatory mediators and oxidative stress parameters in rats. In addition, MPTP also produced significant decrease in brain-derived neurotrophic factor (BDNF) in striatum of rats. However, chronic administration of ceftriaxone (200 mg/kg) has shown significant improvement in motor behavioral deficits and oxidative damage. In addition, Ceftriaxone also attenuated the marked increase of NFκB, TNF-α and IL-1β in MPTP treated rats thus, conferring its neuro-inflammatory property. Further, Ceftriaxone significantly restored the decreased activity of BDNF in striatum of MPTP treated rats. Moreover, pre-treatment of memantine (20 mg/kg) with sub-therapeutic dose of ceftriaxone (100 mg/kg) potentiated the protective effect of ceftriaxone. Furthermore, intra-nigral injection of DHK (200 nmol) with lower dose of ceftriaxone (100 mg/kg) reversed the protective effect of ceftriaxone in MPTP treated rats. The present study concluded that ceftriaxone produce beneficial effect against MPTP induced PD like symptoms rats through glutamatergic pathways.

1. Introduction

Parkinson's disease (PD) is the most common progressive neurodegenerative disease, characterized by bradykinesia, tremor, muscular rigidity and postural instability. Glutamate homeostasis plays a key role in excitotoxicity-related injury in various neurodegenerative disorders. Degeneration of dopaminergic neurons in the nigrostriatal pathway causes over activity of glutamatergic projection to basal ganglia and striatal release of glutamate [1], which triggers serial changes in the basal ganglionic circuitry. Cytotoxic events like mitochondrial dysfunction, excitotoxicity, oxidative stress and neuro-inflammation are prone to doing dopaminergic neuronal cell death in PD [2]. Dysfunction of glutamatergic activity and endogenous anti-oxidative defense system are considered as the two major central events in the process of neuro-inflammation and degeneration of the dopaminergic neurons in PD [3]. Glutamate transporter plays a major role in glutamate clearance in central nervous system which may attenuate adverse behavioral or neurobiological alterations in various neurodegenerative disease models [4]. 90% of the glutamate uptake regulated by glutamate transporter (GLT-1), which is expressed predominantly in astrocytes, regulates the glutamate concentration in the synapse. Increased glutamate accumulation in the synapse results in the excitotoxicity, contributing to oxidative stress and ultimately neuronal cell death [5]. Decreased expression of glutamate transporter protein has been demonstrated in chronic neurodegenerative diseases like PD [4–6]. Excessive release of glutamate can over stimulate *N*-methyl-D-aspartate (NMDA) receptors, causing calcium overload in neurons which lead to neuro-inflammation and neuronal cell death [1,7,8].

MPTP (1-methyl-4phenyl-1, 2, 3, 6-tetrahydropyridine) induced PD is a well-established model of Parkinson's disease producing symptoms similar to human PD [9]. Ceftriaxone, a beta-lactam

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antibiotic, commonly used for its antibacterial property can offer neuroprotective effect on some neurological disorders associated with glutamate excitotoxicity [10]. Ceftriaxone is able to pass freely via the blood brain barrier and has been known to up-regulate the functional expression of glutamate transporter as well as glutamate transporter subtype 1 [11]. Glial glutamate transporters are also known to be associated with the degeneration of dopaminergic neurons in Parkinson's disease due to the glutamate excitotoxicity [12]. It has been reported that dihydrokainate is a specific inhibitor GLT-1 which was employed in several CNS disorders for confirmation of glutamate activity [13]. Increasing evidence suggests that alterations in BDNF expression and signaling might contribute to neurodegeneration. A reduction of BDNF mRNA and protein expression has been consistently reported in the substantia nigra of PD patients [14]. Previously, it has been suggested that GLT-1 up-regulation increase the neurotropic factors in astrocytes and responsible for further survival of dopaminergic neurons [15]. Memantine is an uncompetitive NMDA receptor antagonist, which is well reported for its neuroprotective effect in various neurodegenerative disorders [7]. It acts by blocking the over activation of the glutamate receptors, maintaining the normal physiological glutamate concentration in the synapse [16]. However, the exact role of ceftriaxone in modulating the glutamatergic pathways is still not clear. Therefore, the present study has been aimed to investigate and to explore the neuroprotective effects of ceftriaxone in the presence of glutamate modulators against MPTP induced PD in rats.

2. Materials and methods

2.1. Animals

Young male Wistar rats (230–250 g) bred in the Central Animal House, ISF College of Pharmacy, Moga (India) were used in the study. Animals were acclimated to laboratory conditions at room temperature prior to experimentation. Following surgery, animals were kept under standard laboratory conditions of an under temperature ($22 \pm 1\,^{\circ}C$), relative humidity (60%) and 12 h light/dark cycle with food (Ashirwad Industries, Mohali, India) and water ad libitum in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 09.00 and 15.00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was carried out in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.2. Intranigral administration of MPTP (1-methyl-4phenyl-1, 2, 3, 6tetrahydropyridine)

Surgery was performed as per the previously described protocol [17]. Rats were anaesthetized with ketamine (100 mg/kg, i.p) followed by diazepam (5 mg/kg, i.p.) administration and then positioned in the frame of a stereotaxic apparatus. A midline sagittal incision was made in the scalp to expose bregma. MPTP (1 μmol in 2 μl of saline) was infused bilaterally through a cannula implanted 2.0 mm above the substantia nigra pars compacta (SNPc) through a Hamilton syringe with 30 gauge stainless needle at a rate of 0.7 μl/min at a site with the following coordinates adapted from the rat brain atlas: anterio-posterial: 5.0 mm, middle-lateral: 2.0 mm, ventral depth: 8.0 mm from the bregma, midline, and skull surface respectively [18]. The syringe was allowed to stay in position for next 2 min to prevent back diffusion of the drug. Sham control animals were subjected to the same surgical procedure, but were infused with 2 μl of sterile saline 0.9% instead of MPTP. Immediately after surgery, all rats were administered gentamicin (0.1 ml, 10,000 IU) i.p. to prevent sepsis formation. Special care of the animals was taken during the post-operative period.

2.3. Experiment schedule

2.3.1. Experiment 1

MPTP (Sigma Chemicals Co., St. Louis, MO, USA) was dissolved in normal saline and administered in a volume of (1 μmol/2 μl) on day 1st, 7thand 14th via intranigral injection. Ceftriaxone (Sigma Chemicals Co., St. Louis, MO, USA) (100 and 200 mg/kg) was dissolved in sterile water for injection and administered intraperitoneally (i.p.) for 14 days. Ceftriaxone was started after 14th day of MPTP administration Animals were randomly selected and divided into 5 experimental groups, each group comprises of 7 animals.

1. Sham control group.
2. MPTP (1 μmol/2 μl) + Vehicle (saline)
3. Ceftriaxone (200 mg/kg)
4. MPTP + CFX (100 mg/kg)
5. MPTP + CFX (200 mg/kg)

2.3.2. Experiment 2

2.3.2.1. Administration of dihydrokainate (DHK) and memantine to MPTP induced rats. DHK a selective inhibitor of GLT-1, 20 μl of Dihydrokainate (DHK) (200 nmol) was injected into striatum through intranigral on day 15 after 3doses of MPTP administration. Ceftriaxone and Memantine (20 mg/kg, i.p.) treatment was started after 15th day of DHK administration in MPTP injected rats. Animals were randomly selected and divided into 4 experimental groups, each group comprises of 7 animals.

1. MPTP + MEM (20 mg/kg)
2. MPTP + MEM (20 mg/kg) + CFX (100 mg/kg)
3. MPTP + DHK (200 nmol)
4. MPTP + DHK (200 nmol) + CFX (100 mg/kg)

Different behavioral performances and biochemical analysis has been carried out as per scheme in Fig. 1.

2.3.2.2. Measurement of body weight. Animal body weights were recorded on 1st and 14th day after surgery and on 21st and 28th day of the experiment, for calculating the percent change in body weight.

2.4. Behavioral assessments

2.4.1. Locomotor activity

The locomotor activity was assessed on day 1, 7 and 14 days before drug treatment and on 21st and 28th day after drug treatment using an actophotometer (Medicraft photo actophotometer, Model no: 600 M-6D, INCO, Ambala). The motor activity was detected by infrared beams (6 beams which were placed at the distance of 6 cm each) above the floor of the testing chamber, the interruption of beam on X or Y axis generated an electric impulse which was presented on digital counter to record the locomotor activity. It measures the spontaneous and indicated activity with digital counter. The units of the activity counts were arbitrary and based on the beam breaks by movement of the rat both rearing and ambulatory. Five min habituation period was given to the animals for exploring the apparatus. Each animal was observed over a period of 10 mins and expressed as counts per 10 min [19].